

***CYP2C19* genetics in fatal carisoprodol intoxications**

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0. Abstract

Introduction: Carisoprodol, a frequently used muscle relaxant, can cause potentially fatal intoxications. Conversion to its active metabolite meprobamate is almost solely mediated by cytochrome P450 2C19 (CYP2C19), and mutations in this enzyme could have significant effects on serum concentrations. The objective of this study was to investigate the role of CYP2C19 genetics in mortalities due to carisoprodol intoxication.

Methods: The frequencies of CYP2C19 variant alleles were compared between the study group (n 0 75) and two control groups, i.e. (1) deaths where carisoprodol was detected in the blood of the deceased, but intoxication was not the cause of death (control group A, n 0 38), and (2) a healthy population not using carisoprodol (control group B, n 0 185). In the study group and control A, the concentrations of carisoprodol and meprobamate were compared between the different genotype subgroups.

Results: The variant allele frequencies of CYP2C19 did not differ significantly between the study group and control groups. Moreover, no statistically significant difference in the concentrations of carisoprodol and meprobamate between the different genotype subgroups was found.

Conclusions: This study finds no evidence for an important association between CYP2C19 genetics and mortality risk of carisoprodol. Other factors, such as co-administration with other drugs, likely play a more important role.

1. Introduction

Carisoprodol is a centrally acting muscle relaxant used for acute back pain [1], but there are also reports of abuse [2-4] and deaths [5-7] attributed to the drug. We have previously published a report on 98 fatal carisoprodol intoxications and showed that the full blood carisoprodol concentrations were lower than previously published [8, 9], thus indicating that carisoprodol has a more narrow therapeutic index than assumed earlier [10].

Carisoprodol has an unknown mechanism of action, but reveals effects like tachycardia and dizziness [11]. It is metabolised almost solely via the genetic polymorphic enzyme *CYP2C19* to the active metabolite meprobamate, which has barbiturate-like properties. The *CYP2C19* gene comprises different alleles, where the *1 (wild type) allele encodes normal activity, and the *2, 3 and 4 variant alleles codes for no activity ('deficient' variant alleles). The *CYP2C19**17 variant allele has been attributed to increased enzyme activity but the phenotypic impact has been variable in different studies [12].

In the case of carisoprodol, it is likely that carriers of deficient *CYP2C19* variant alleles obtain higher concentrations of the parent drug, which may imply an increased risk of intoxications when considering carisoprodol's narrow therapeutic index. On the other hand, it is a possibility that carriers of the *CYP2C19**17 variant allele obtain higher concentrations of the toxic metabolite meprobamate. The aim of the present study was therefore to investigate if presence of *CYP2C19* variant alleles is associated with increased risk of fatal intoxications with carisoprodol.

2. Materials and methods

The Norwegian Institute of Public Health, Division of Forensic Medicine and Drug Abuse Research routinely analyses for drugs in blood samples from 90% of the forensic autopsies performed in Norway. The material in the present study constitutes all such cases in the period January 1st 2000 to December 31st 2003 in which carisoprodol and/or its metabolite meprobamate were detected. Meprobamate was rarely used as a drug as such in Norway, and when meprobamate was detected alone, the finding was considered to represent intake of carisoprodol. In the following, we therefore refer to the detection of carisoprodol when either carisoprodol or its metabolite was detected.

After approval from the Norwegian Data Inspectorate, the official cause of death was recorded, and the cases were divided into those who died from intoxication with carisoprodol (study group) and a control group (control A) of those who died from other causes, but with carisoprodol detected in blood [10]. *CYP2C19* genotyping was carried out in all cases where a post mortem blood sample was still available. The study was approved by the regional Committee for Medical and Health Research Ethics as well as the Norwegian Higher Prosecuting authority, which stand as the official owners of the forensic material.

Carisoprodol and meprobamate were analysed in samples of post mortem full blood using a liquid chromatography-mass spectrometry method [10]. *CYP2C19* genotyping was carried out using validated and certified Taqman-based real-time PCR assays developed for routine analysis at Centre for Psychopharmacology, Diakonhjemmet Hospital, Norway. *CYP2C19* genotyping included analysis of the *2, *3 and *17 variant alleles. Absence of these variant alleles was interpreted as presence of the wild type (*1) allele.

The *CYP2C19* allele frequencies and genotype frequencies in the study group were compared with two control groups, i.e control A (described above) and previously published data from 185 healthy Swedes who were not users of carisoprodol (control B) [13]. This is shown in table 1. Detailed genotype data from control B were kindly provided via e-mail by Eleni Aklillu, Department of laboratory medicine at Karolinska University Hospital, Stockholm, Sweden. Genotype and variant allele frequencies were compared using Fisher's exact test. Differences in frequencies between the subgroups were calculated as odds ratios (OR) with 95% confidence intervals. The blood concentrations of carisoprodol, meprobamate and the ratio meprobamate/carisoprodol were compared between the different genotype groups using Kruskal-Wallis non-parametric rank analysis of variance to establish an overall difference between the *CYP2C19* genotype groups. Eventually, Dunn's post test was applied for multiple comparisons. GraphPad Prism version 4 was used as software for statistical analyses.

3. Results

The different groups and number of subjects are shown in table 1. In the study group, the cases were classified according to the importance of carisoprodol, using previously published criteria [10]. Three cases involved carisoprodol only, while 27 cases involved other drugs, but not in fatal concentrations. Carisoprodol was therefore considered the most important intoxicating drug. In the final 45 cases, additional drugs were present in fatal concentrations, and carisoprodol could therefore be less important as an intoxicating agent (table 1).

The *CYP2C19* allele frequencies for the *1, *2 and *17 alleles in the study group, control A and control B are shown in table 2. There were no detected *3 alleles in the study group or control A. This allele is also previously described to be very rare in the Caucasian population [13]. Variant allele frequencies of the *1, *2 or *17 alleles did not differ significantly between the study group and any of the control groups (table 2).

The *CYP2C19* genotype frequencies in the study group and control groups are shown in table 3. Genotype frequencies did not differ significantly between the study group and any of the control groups (table 3), except from a lower frequency of the *1/*2 genotype in the study group compared to control A (OR 0.33, p=0.03).

The allele frequencies and genotype frequencies were also compared excluding the cases from the study group where carisoprodol was less important as an intoxicating agent. When using only the study group cases in which carisoprodol was the only, or most important intoxicating agent (n=30), no difference in allele frequencies or genotype frequencies was seen compared to control A or control B.

There were no statistically significant differences in concentrations of carisoprodol or meprobamate (study group and control A together, $n=113$) between the different genotype groups ($p>0.05$ for trend for both carisoprodol and meprobamate). There was no statistically significant difference in the meprobamate/carisoprodol ratio between the different genotype groups ($p>0.05$ for trend). If concentrations of carisoprodol, meprobamate and the ratio meprobamate/carisoprodol were assessed within the study group and control A, respectively, there were no statistically significant difference in concentrations or ratio between the different genotype groups ($p>0.05$ for trend) (figure 1).

4. Discussion

In this study, we found no significant differences in frequencies of *CYP2C19* variant alleles among 75 subjects who died of intoxication with carisoprodol compared to i) those who died from other causes than intoxication, but with carisoprodol detected in blood, or ii) healthy individuals. This study therefore failed to find evidence for an important association between *CYP2C19* genetics and the risk of fatal toxicity induced by carisoprodol. With regard to the single significant difference in genotype frequency, i.e. a lower proportion of *CYP2C19**1/*2 carriers in the study group compared to control A, we consider this to be a coincidence as the frequency of this genotype did not differ between the study group and healthy subjects.

When interpreting studies yielding negative results, statistical power is an important issue to consider. Given the allele frequency of *CYP2C19**1 in healthy controls (0.64 in control group B), post hoc calculations showed that the present study would have an 80% power to detect a significant difference if *1 allele frequency was below 0.45 or above 0.81 in the study group (n=75). For the *2 allele, the corresponding numbers were 0.04 and 0.32. Thus, it is reasonable to interpret that the sample size of the study was sufficient to detect clinically relevant differences in allele frequencies of the *1 and *2 alleles between study group and control B, except from decreased presence of the *2 allele in the study group.

The relation between *CYP2C19* genotype and deaths from carisoprodol has never been studied before. However, a small number of previous studies have failed to find a relation between *CYP2C19* genotype and fatal intoxications, regardless of drug detected [14, 15]. This was strengthened by the present study. The limited impact of CYP genetics on risk of fatal drug-induced toxicity could have several explanations. Most important, a vast majority of the

intoxications in the present study involved multiple misuse agents, which are likely to potentiate each others toxic effects.

We did not find concentration differences of carisoprodol and meprobamate between the different *CYP2C19* genotype groups. This is in contrast to what have been observed in living subjects in former studies. For carisoprodol, a 30% longer terminal half life and a 40% higher area under the curve have previously been reported in **1/*2* subjects [11, 16]. However, a post mortem material is much less controlled with respect to dose ingested and presence of steady state level. Also, it is possible that post mortem redistribution may change blood concentrations after death, a phenomenon which is likely to be more pronounced for meprobamate than carisoprodol, considering their different volumes of distribution. Together, this could have masked the relatively moderate expected concentration differences between **1/*1* and **1/*2* subjects. The pharmacokinetic differences between homozygous wild type carriers and **2/*2* subjects have previously been reported to be more pronounced [11], but unfortunately we had too few subjects with the latter genotype to compare these subgroups.

The strength of this study was the inclusion of a relatively large number of fatal toxicity cases and the fact that all subjects died from intoxication with carisoprodol, a known *CYP2C19* substrate. Moreover, another strength of the present study was the use of the official cause of death, which is set after all examinations are completed. The most important weakness of the present study was the involvement of other drugs than carisoprodol in most of the cases, a well known problem in forensic medicine [9]. When analysing the data only using the cases where carisoprodol was the only, or most important intoxicating drug (n=30), no difference to the control groups were seen, neither in allele frequencies nor genotype frequencies. Such reduction of the study group sample size would reduce the power and demand larger

differences in allele frequencies before it would yield statistically significant results. Also, the control group from the normal population was not users of carisoprodol.

In conclusion, this study suggests that *CYP2C19* genetics is not an important risk factor for carisoprodol-associated mortality. Other factors, such as dynamic interactions with other drugs, probably play a more important role in fatal intoxications.

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5. Acknowledgements

The authors are grateful to Eleni Aklillu for providing genotype frequencies in control group B and to Asbjørg Christophersen and Ritva Karinen for organizing the blood samples. We also thank Linda Uthus and Ida Mari Haugom for genotyping the post mortem samples.

Table 1. The different groups of subjects in the present study

Group		Role of carisoprodol	n
Study group	Deaths from intoxication	Carisoprodol only	3
		Carisoprodol most important	27
		Carisoprodol less important	45
Control A	Deaths from other causes (e.g violent deaths)	Carisoprodol detected	38
Control B	Healthy individuals	Not users of carisoprodol	185

Table 2. *CYP2C19* allele frequencies and odds ratios (OR, 95% CI) in the study group (fatal intoxications) versus the respective control groups. Control A = deaths from other causes, control B = normal population.

	Study group	Control A	OR (95% CI)	Control B	OR (95% CI)
n	75	38		185	
*1	0.69	0.67	1.11 (0.61-2.00)	0.64	1.27 (0.84-1.90)
*2	0.11	0.18	0.57 (0.26-1.22)	0.16	0.67 (0.39-1.20)
*17	0.19	0.15	1.42 (0.66-3.02)	0.20	0.96 (0.59-1.55)

Table 3. *CYP2C19* allele frequencies and odds ratios (OR, 95% CI) in the study group (fatal intoxications) versus the respective control groups. Control A = deaths from other causes, control B = normal population. ¹Significant value (p=0.03).

	Study group	Control A	OR (95% CI)	Control B	OR (95% CI)
<u>n</u>	<u>75</u>	<u>38</u>		<u>185</u>	
<u>*1/*1</u>	<u>35 (0.47)</u>	<u>15 (0.39)</u>	<u>1.34 (0.61-2.97)</u>	<u>76 (0.41)</u>	<u>1.26 (0.73-2.15)</u>
<u>*1/*2</u>	<u>11 (0.15)</u>	<u>13 (0.34)</u>	<u>0.33 (0.13-0.84)¹</u>	<u>34 (0.18)</u>	<u>0.76 (0.36-1.60)</u>
<u>*2/*2</u>	<u>1 (0.01)</u>	<u>0 (0.00)</u>	<u>1.55 (0.06-39.00)</u>	<u>7 (0.04)</u>	<u>0.34 (0.04-2.84)</u>
<u>*2/*17</u>	<u>4 (0.05)</u>	<u>1 (0.03)</u>	<u>2.09 (0.23-19.34)</u>	<u>12 (0.07)</u>	<u>0.81 (0.25-2.61)</u>

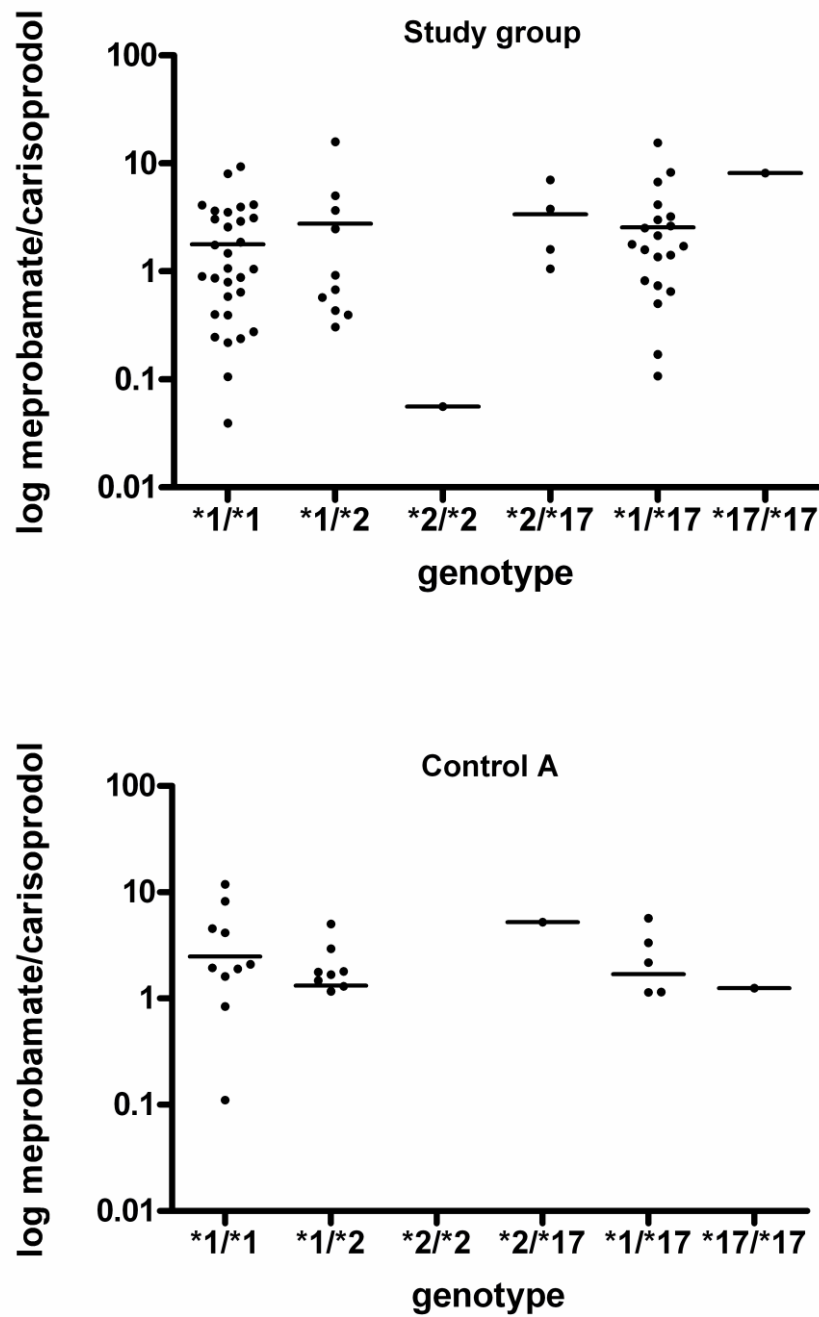


Figure 1. Log ratio meprobamate/carisoprodol in the different *CYP2C19* genotype groups in study group (above) and control A (below).